SUPPLEMENTAL METHODS

Reagents

Tamoxifen was purchased from Toronto Research Chemicals (Toronto, Canada). Sunflower seed oil, bovine serum albumin, and paraformaldehyde were from Sigma (St. Louis, MO). 3'-sialyllactose-APD HAS and 6'-sialyllactose-APD HAS compounds were purchased from Accurate Chemical and Scientific Corporation (Westbury, NY). Brucella broth was purchased from Bio-World (Dublin, OH). Fetal bovine serum was from Thermo Fisher Scientific (Houston, TX). For selective media plates, tryptic soy agar was from Remel (Lenexa, KS), sheep blood from Colorado Serum Company (Denver, CO), amphotericin from Corning (Tewksbury, MA), bacitracin from Thermo Fisher Scientific, vancomycin and nalidixic acid from Sigma. AlexaFluor-594-conjugated phalloidin (1:100) was from Thermo Fisher Scientific. Rabbit anti-Hp (1:1000) was kindly provided by Manuel Amieva (Stanford University), and rabbit anti-gastric intrinsic factor (GIF; 1:10,000) was kindly provided by David Alpers (Washington University). *Maackia amurensis* lectin (MAA; labels sLe^x; 1:100) was from EY Laboratories (San Mateo, CA), mouse anti-Lewis b (Le^b; 1:200) was from BioLegend (San Diego, CA), goat anti-ezrin (1:100) was from Santa Cruz Biotechnology (Dallas, TX).

Helicobacter pylori (*H pylori*) Growth and Infection

The mouse-adapted, wild-type pre-mouse Sydney strain, PMSS1[1], was kindly provided by Rick Peek (Vanderbilt University). The NSH57 strain and the *babA*-, *sabA*-, and *babA*/*sabA*-deficient isogenic mutants[2] were kindly provided by Nina Salama (University of Washington). For growth of the PMSS1 strain, frozen stocks were plated on tryptic soy agar containing 5% sheep blood (Life Technologies, Carlsbad, CA) and maintained at 37℃ and 5% CO_2 for 2-3 days, passaged once for an additional 1-2 days, then transferred to liquid culture (Brucella broth, 10% heat-inactivated fetal bovine serum, 20 µg/mL vancomycin) and grown for 12-18 hours at 37℃ and

5% CO_2 with shaking (120 rpm). Growth and initial characterization of the NSH57 strain and the isogenic mutants have been previously described[2]. Wild-type C57/BL-6 mice, aged 6-8 weeks, were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were fasted for 4-6 hours prior to oral gavage with 200 µL of an overnight *H pylori* culture, then fasted for an additional 1-2 hours after infection. Prior to infection, an aliquot of the overnight *H pylori* culture was serially diluted, plated on tryptic soy agar plates containing 5% sheep blood, and incubated at 37℃ and 5% CO_2 for 5-7 days to determine the inoculum dose. For all infections and treatments, both male and female littermates were used and analyzed.

***In Vivo* Immunofluorescence**

For chronically infected mouse stomachs, mice were sacrificed by cervical dislocation, and their stomachs were excised. After removing the forestomach, stomachs were cut open along the lesser curvature, and any residual food was gently scraped away. Stomachs were pinned out in 4% PFA and fixed overnight at 4℃. The next day, st omachs were washed in phosphate-buffered saline (PBS; pH 7.4) and kept in 70% ethanol until further processing. Stomachs were cut in longitudinal sections that were arranged in 2% agar in a tissue cassette and underwent routine paraffin processing. 5-µm sections were cut and mounted on glass slides. Prior to staining, sections were subjected to a standard deparaffinization and rehydration protocol. Slides were boiled for 10 minutes in 10 mM sodium citrate (pH 6.0), then blocked and processed for immunofluorescent staining as described in the Methods section.

Assessing Glandular Distribution of *H pylori In Vivo*

For *in vivo H pylori* infection experiments, paraffin-embedded tissue sections were stained as described above. Images were captured as TIFF files with a Zeiss Axiovert 200 microscope with Axiocam MRM camera and with an Apotome optical sectioning filter. Using ZEN software (Zeiss, San Diego, CA), the total length of each individual *H pylori* -colonized corpus gland was

measured. The percent distance along the gland axis was determined by calculating the distance of *H pylori* from the top of the gland as a percentage of the total gland length. For glands containing multiple bacteria, the distance of the deepest *H pylori* bacterium was counted.

Immunohistochemistry of Human Gastric Specimens

Human gastric biopsy samples from patients undergoing upper endoscopy, or gastric resection specimens from patients with known gastric adenocarcinoma or undergoing bariatric sleeve gastrectomies, were obtained with approval from the Institutional Review Board of Washington University School of Medicine. Paraffin-embedded (5-μm) sections of human gastric specimens were deparaffinized and rehydrated according to routine paraffin processing protocols. Endogenous peroxidase activity was quenched with 1.5% H_2O_2 in methanol for 15 minutes at room temperature. Slides were boiled for 10 minutes in 10 mM sodium citrate (pH 6.0), then blocked for 2 hours at room temperature in blocking buffer (see Methods) in a humidity chamber. Slides were subsequently blocked using the Avidin/Biotin blocking kit (Vector Laboratories, Burlingame, CA), according to the manufacturer's protocol. For sLe^x staining, slides were incubated in biotinylated MAA (1:100; EY Laboratories) overnight at 4℃ and washed with PBS the next day. Signal was amplified using the VectaStain Elite ABC kit (Vector Laboratories) and developed using 3,3'-diaminobenzidine tetrahydrochloride (Thermo Fischer Scientific). Slides were counterstained with hematoxylin according to routine staining protocols. Staining against human MIST1, CBP1, and TFF2 has been previously described[3].

Competitive Inhibition of *H pylori* Binding *In Situ*

Free-floating thick sections were blocked with PBS containing 3% bovine serum albumin overnight. The next day, an overnight culture of *H pylori* was diluted in pre-warmed PBS or varying concentrations of 3'- or 6'-sialyllactose diluted in PBS. The sections were infected and

processed for indirect immunofluorescence as described in the *In Situ H pylori* Adherence Assay section.

Determining intra-gastric pH

C57/BL-6 wild-type mice (male and female littermates) were housed in a specified pathogen-free facility under a 12-hour light cycle. Mice were intra-peritoneally injected with either vehicle or HD-Tam (5 mg/20 g body weight) for 2 days prior to sacrifice. For omeprazole treatment, mice were orally gavaged daily with omeprazole (1.5 mg/20 g body weight resuspended in saline) for 7 days prior to sacrifice. Mouse stomachs were excised, the forestomach was removed with a razor blade, and the small intestine was separated from the stomach, approximately 1 cm distal to the pylorus. A gavage needle was inserted into the pylorus, and the stomach was flushed with 500 µL of sterile PBS (pH 7.4) into a 1.5-mL Eppendorf tube. The samples were briefly centrifuged, and the supernatant was transferred to a new 1.5-mL Eppendorf tube. The pH of the sample was measured using a micropH electrode (Fisher Scientific).

Neuraminidase treatment of thick gastric corpus sections

Free-floating, 100-µm sections from the gastric corpus of HD-Tam-treated mice were incubated at 37℃ and 5% CO_2 overnight in either 50 mM sodium acetate (pH 5) or type VI neuraminidase (Sigma; 25 U/mL in sodium acetate, pH 5). The next day, sections were washed three times (5 minutes per wash) in PBS at room temperature, then incubated with *H pylori* (OD_{600} = 0.02) for 1 hour in PBS containing 3% BSA. Sections were washed, fixed, and processed for immunofluorescence, as described in the Methods section.

SUPPLEMENTAL REFERENCES

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2. Talarico S, Whitefield SE, Fero J, et al. Regulation of Helicobacter pylori adherence by gene conversion. Mol Microbiol 2012;84:1050-61.
3. Lennerz JK, Kim SH, Oates EL, et al. The transcription factor MIST1 is a novel human gastric chief cell marker whose expression is lost in metaplasia, dysplasia, and carcinoma. Am J Pathol 2010;177:1514-33.
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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1.

Treatment with HD-Tam causes a reversible loss of parietal cells. (A) 100-μm gastric corpus sections were prepared from mice treated with vehicle/HD-Tam or allowed to recover from HD-Tam injury, as described in Methods. Representative images demonstrate that HD-Tam treatment acutely and reversibly induces parietal cell loss (labeled with anti-ezrin antibody in red). Mucous neck cells are highlighted in green. Scale bars, 50 μm. (B) Treatment of mice with HD-Tam blurs the gross morphologic distinction between gastric corpus and antrum. Mice were treated with vehicle/HD-Tam or allowed to recover from HD-Tam injury (recovery) prior to sacrifice. Stomachs were excised, the forestomach was removed, and the stomachs were pinned out in PBS. A representative stomach is shown for each treatment condition. Note the mucosal pallor in the HD-Tam-treated stomach compared to the vehicle-treated or recovered stomachs. (C) Treatment with HD-Tam increases intra-gastric pH within the range of omeprazole treatment. Male and female mice were treated with vehicle/HD-Tam for 2 days or with daily omeprazole gavage for 7 days prior to sacrifice. Intra-gastric pH was measured, as described in Supplemental Methods. Data represent the mean intra-gastric pH for each treatment condition. Each data point represents a biological replicate. Means between multiple

treatments were compared using one-way analysis of variance with the Dunnett's multiple comparisons test to determine significance. * $p < 0.05$; n.s., not significant ($p > 0.05$). The difference in intra-gastric pH between omeprazole- and vehicle-treated mice showed a trend toward statistical significance ($p = 0.06$).

Supplemental Figure 2.

***H pylori* binds terminal sialic acid residues on SPEM glands.** 100-μm gastric corpus sections from HD-Tam-treated mice were incubated with either vehicle or neuraminidase (NA) prior to incubation with *H pylori* (see Supplemental Methods). Treatment with NA, which cleaves terminal sialic acid residues, impairs the ability of *H pylori* (green) to access deeper regions of SPEM glands. Cleavage of sialic acid residues results in decreased binding of *Maackia amurensis* lectin (MAA; red) to its receptor, sLe^x. In the absence of NA (vehicle), MAA recognizes sLe^x along the length of the SPEM gland. Images in the left panels represent isolated *H pylori* signal from the merged images in the corresponding right panels. Blue, nuclei. Scale bars, 50 μm. Hp, *H pylori*.

Supplemental Figure 3.

Omeprazole treatment does not induce SPEM.

Mice were intra-peritoneally injected with either vehicle/HD-Tam for 2 days or orally gavaged for one week with omeprazole prior to sacrifice. Stomachs were excised and processed for immunofluorescent staining, as described in Methods. Representative images are shown. Unlike HD-Tam treatment, which induces co-expression of the chief cell marker GIF (red) and the neck cell marker GSII (green) at the base of corpus glands (changes consistent with SPEM), omeprazole treatment does not induce this co-localization, similar to vehicle treatment. Scale bars, 50 μm.

Supplemental Figure 4.

Identification of human gastric biopsy samples for sLe^x staining.

A total of 221 separate human gastric biopsy samples were obtained from upper endoscopic procedures performed at the Washington University School of Medicine over two years. Of these, only biopsies from the gastric corpus or fundus were considered for further analysis. Positivity for *H pylori* infection was determined by Steiner stain. A total of 5 gastric biopsy samples from patients with confirmed Hp infection were used for additional staining. Hp, *H pylori*.

Supplemental Figure 5.

The pattern of sLe^x in humans correlates with SPEM.

Human gastric biopsy samples from the corpora of *H pylori* -infected patients demonstrating varying gastric histopathology were stained against sLe^x as well as chief cell and mucous neck cell markers. (A) The sLe^x staining pattern (i, ii) is restricted to the pit region in a 46 year-old patient with chronic, non-atrophic gastritis. (iii, iv) Note the absence of SPEM in this biopsy, as evidenced by the lack of co-expression of the mucous neck cell marker, TFF2 (red), with the chief cell-specific transcription factor, MIST1 (brown nuclei). (B) sLe^x staining (i, ii) can be observed in the pits and bases (marked by asterisk) of SPEM glands in a 75 year-old patient with chronic atrophic gastritis. (iii, iv) SPEM glands are highlighted by the co-expression of TFF2 (red) and MIST1 (brown nuclei). The MIST1-positive extra-epithelial cells surrounding the gland bases (iv) represent plasma cells, which have been shown to strongly and specifically express MIST1[4]. For (A-B), TFF2, trefoil factor 2. (C) In this 71 year-old patient with chronic atrophic gastritis and focal intestinal metaplasia, sLe^x staining (i, ii) can be observed in the pits and bases of SPEM glands but is excluded from areas of intestinal metaplasia, denoted by arrowheads in (i). sLe^x highlights the bases of SPEM glands, characterized by the co-expression of TFF2 (red) and the chief cell marker, CBP1 (brown; iii, iv). In (iv), SPEM cells

(arrowheads) can be seen adjacent to a normal gland base. TFF2, trefoil factor 2; CBP1, carboxypeptidase 1. For (A-C), scale bars in sub-panels (i) and (iii) are 200 μm, and scale bars in sub-panels (ii) and (iv) are 50 μm. (D) Representative image from a gastric resection specimen of a 69 year-old patient with a mucinous gastric adenocarcinoma. This region of SPEM adjacent to the tumor demonstrates foveolar hyperplasia and tortuous SPEM glands (arrowheads in ii) highlighted by sLe^x expression. Dotted lines outline the interface between the gastric epithelium and the muscularis mucosa. Scale bars, 500 μm. For (A-D), sub-panels (ii) and (iv) represent magnified images of sub-panels (i) and (iii), respectively. All images in (A-D) are from patients who had confirmed *H pylori* infection by Steiner stain.

Supplemental Table 1.

Summary of human gastric sample analysis.

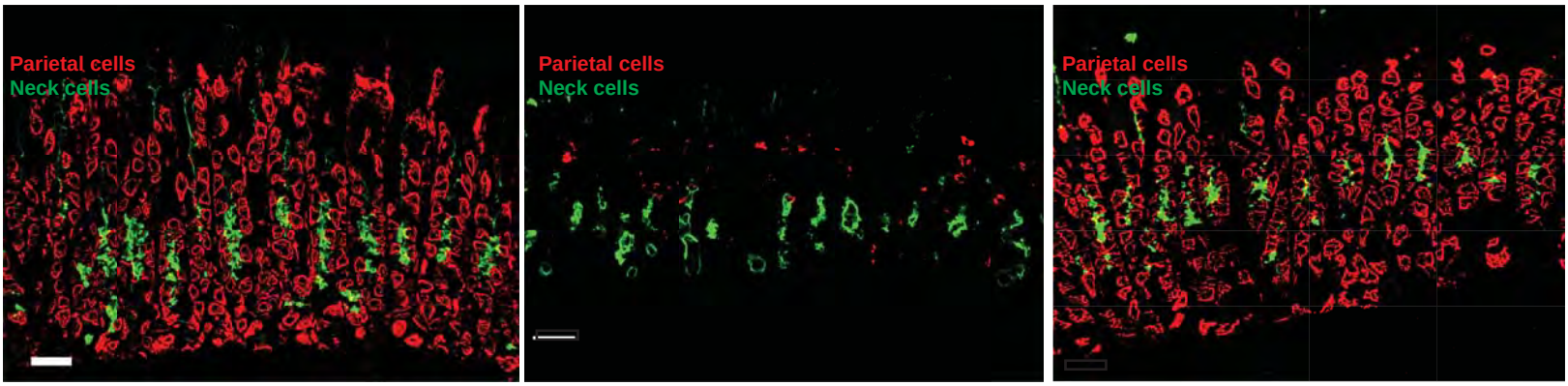
Demographic and histopathologic data are presented for all of the human gastric samples that were stained. *H pylori* positivity was determined by Steiner stain of gastric biopsies. The histopathologic diagnosis was determined prior to staining for sLe^x, MIST1, TFF2, or CBP1. The pattern of sLe^x staining is described for each biopsy, and representative images are presented in Supplemental Figure 5.

Supplemental Movie.

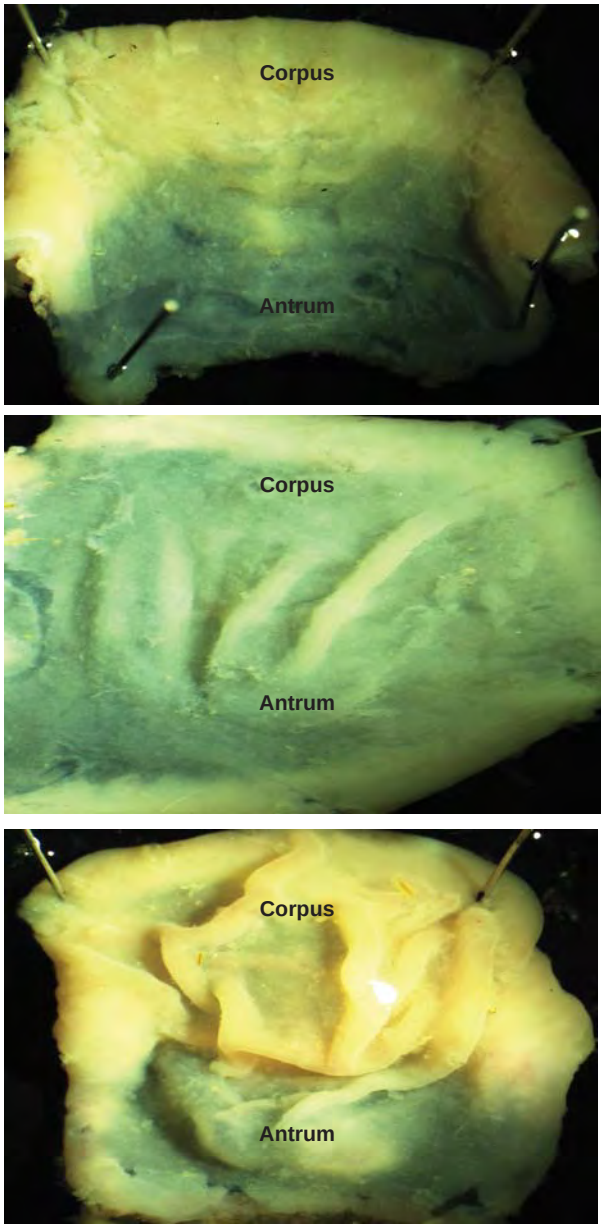
Identification of intra-glandular *H pylori*.

Three-dimensional reconstruction of an isolated SPEM gland containing *H pylori* (green). Red highlights actin staining. Bacteria located outside of the gland are labeled with arrowheads.

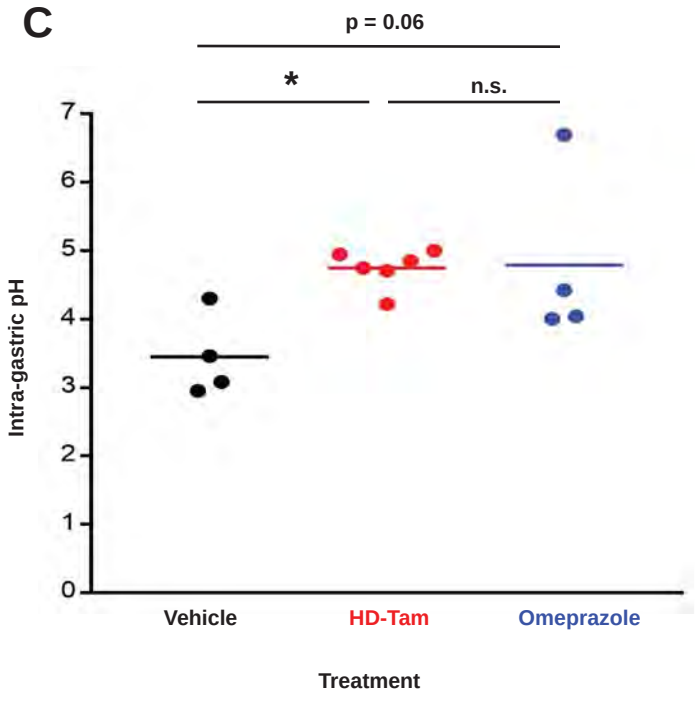
Patient	Age (years)	Diagnosis	*Helicobacter pylori* positive?	Pattern of sLe^x staining
1	67	Acute/chronic gastritis	Yes	Restricted to bases of SPEM glands.
2	59	Chronic atrophic gastritis	Yes	Highlights foveolar hyperplasia in SPEM glands; scant staining in bases of SPEM glands.
3	70	Acute gastritis (minimal)	Yes	Highlights foveolar hyperplasia in SPEM glands; scant staining in bases of SPEM glands.
4	46	Chronic gastritis	Yes	Restricted to pit/foveolar epithelium; no SPEM glands appreciated.
5	75	Chronic atrophic gastritis	Yes	Highlights foveolar hyperplasia and bases of SPEM glands.
6	50	Acute gastritis (severe)	No	Highlights foveolar hyperplasia; scant staining of gland bases.
7	71	Chronic atrophic gastritis with focal intestinal metaplasia	No	Highlights foveolar hyperplasia and bases of SPEM glands; staining not seen in areas of intestinal metaplasia.
8	Unknown	Sleeve gastrectomy (normal)	No	Restricted to pit/foveolar epithelium; no SPEM glands appreciated.
9	36	Sleeve gastrectomy (normal)	No	Restricted to pit/foveolar epithelium; no SPEM glands appreciated.
10	69	Gastric adenocarcinoma	No	Stains mucinous tumor; highlights foveolar hyperplasia and bases of SPEM glands adjacent to tumor.

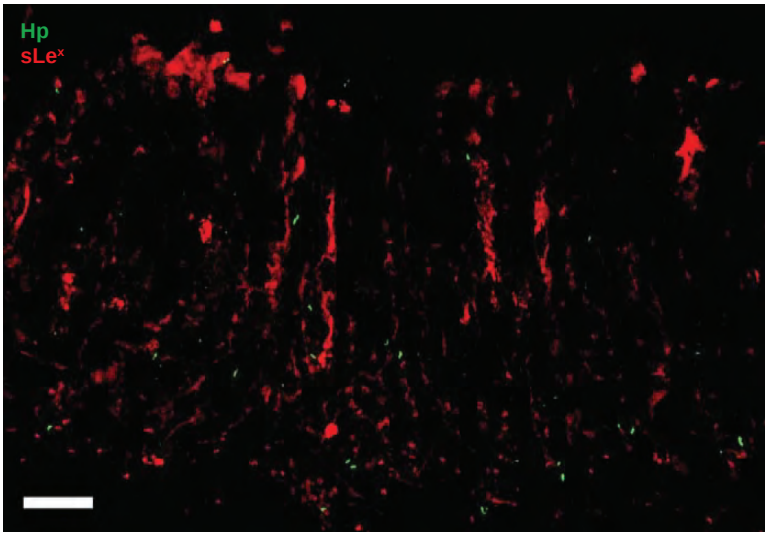

Parietal cells
Neck cells
Parietal cells
Neck cells
Parietal cells
Neck cells

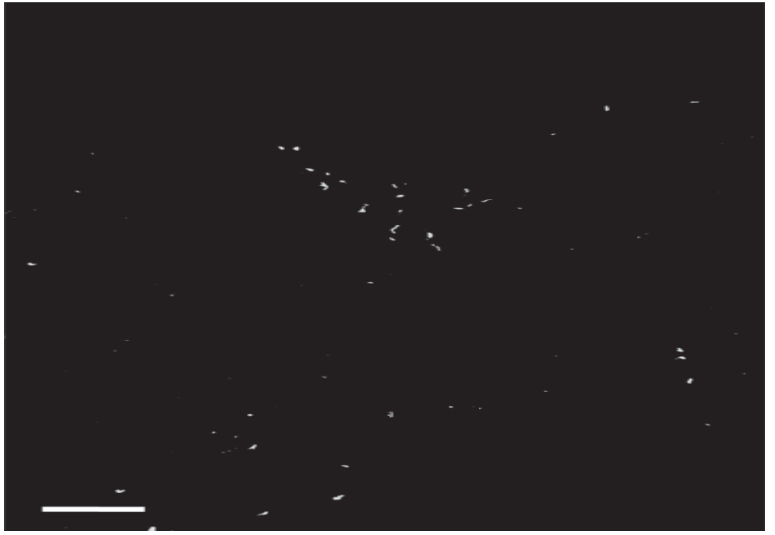
B

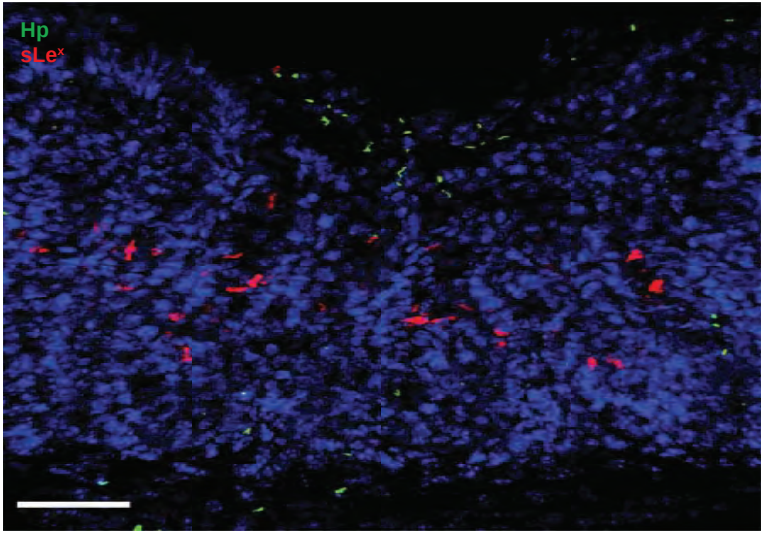

Corpus
Antrum
Corpus
Antrum
Corpus
Antrum

C


p = 0.06
*
n.s.
Intra-gastric pH
0
1
2
3
4
5
6
7
Vehicle
HD-Tam
Omeprazole
Treatment


Hp
sLe^x

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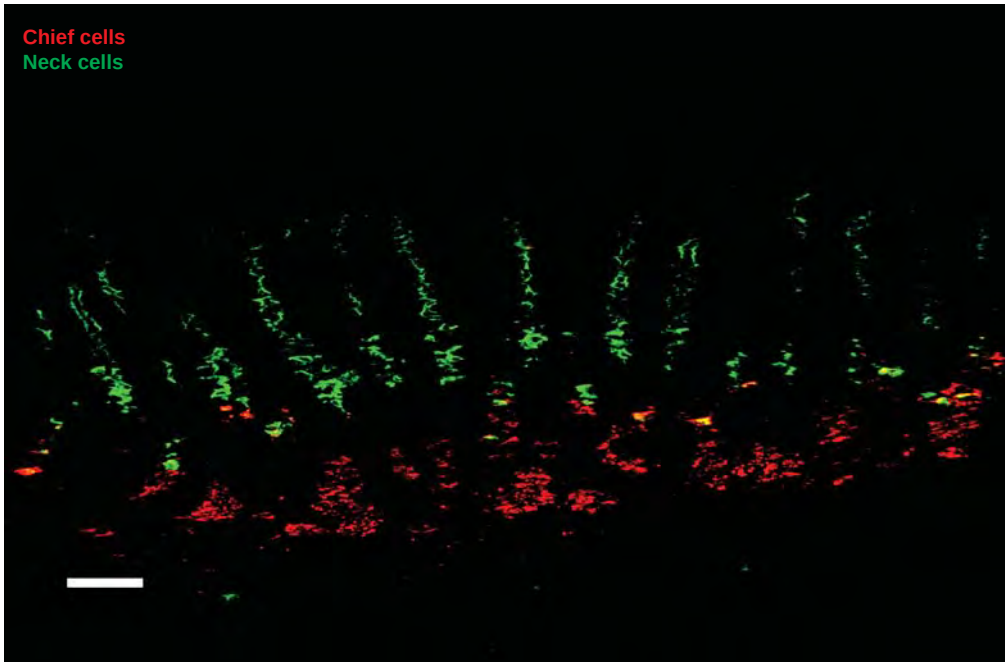
sLe^x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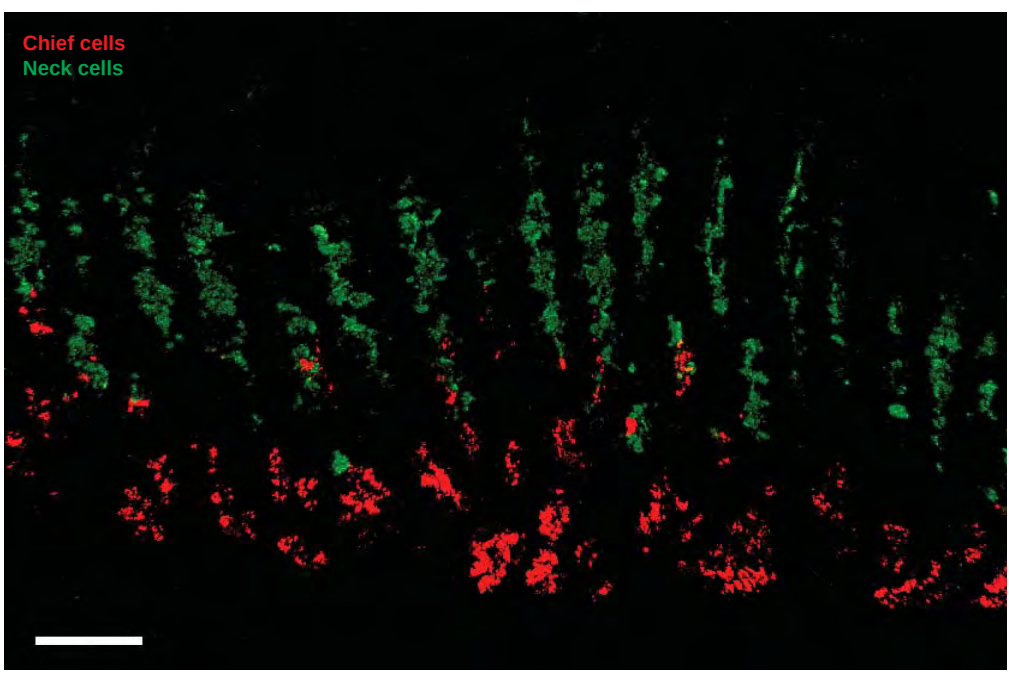
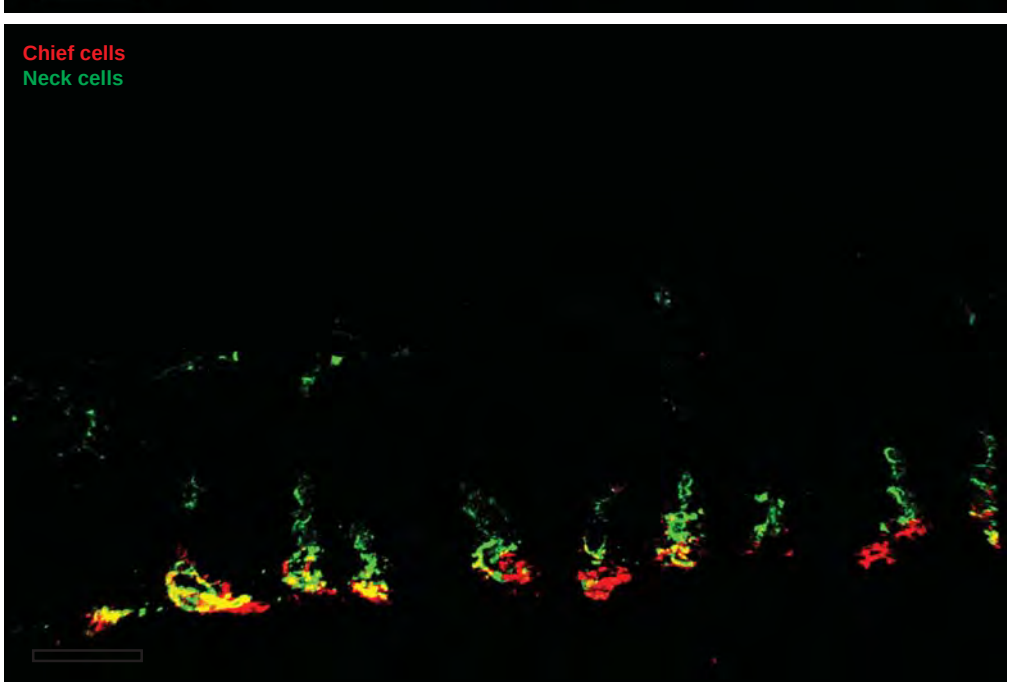
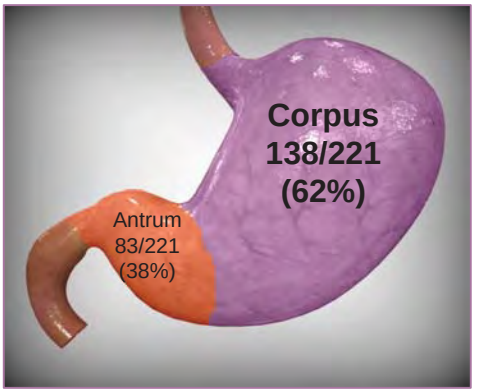
Neck cells


Chief cells
Neck cells


Chief cells
Neck cells



Chronic Atrophic Gastritis
(SPEM; n = 36)

Chronic gastritis
(non-atrophic; n = 57)

Other
(acute gastritis, reactive gastropathy,
no histologic abnormalities; n = 45)

Hp-positive?
(Steiner stain)

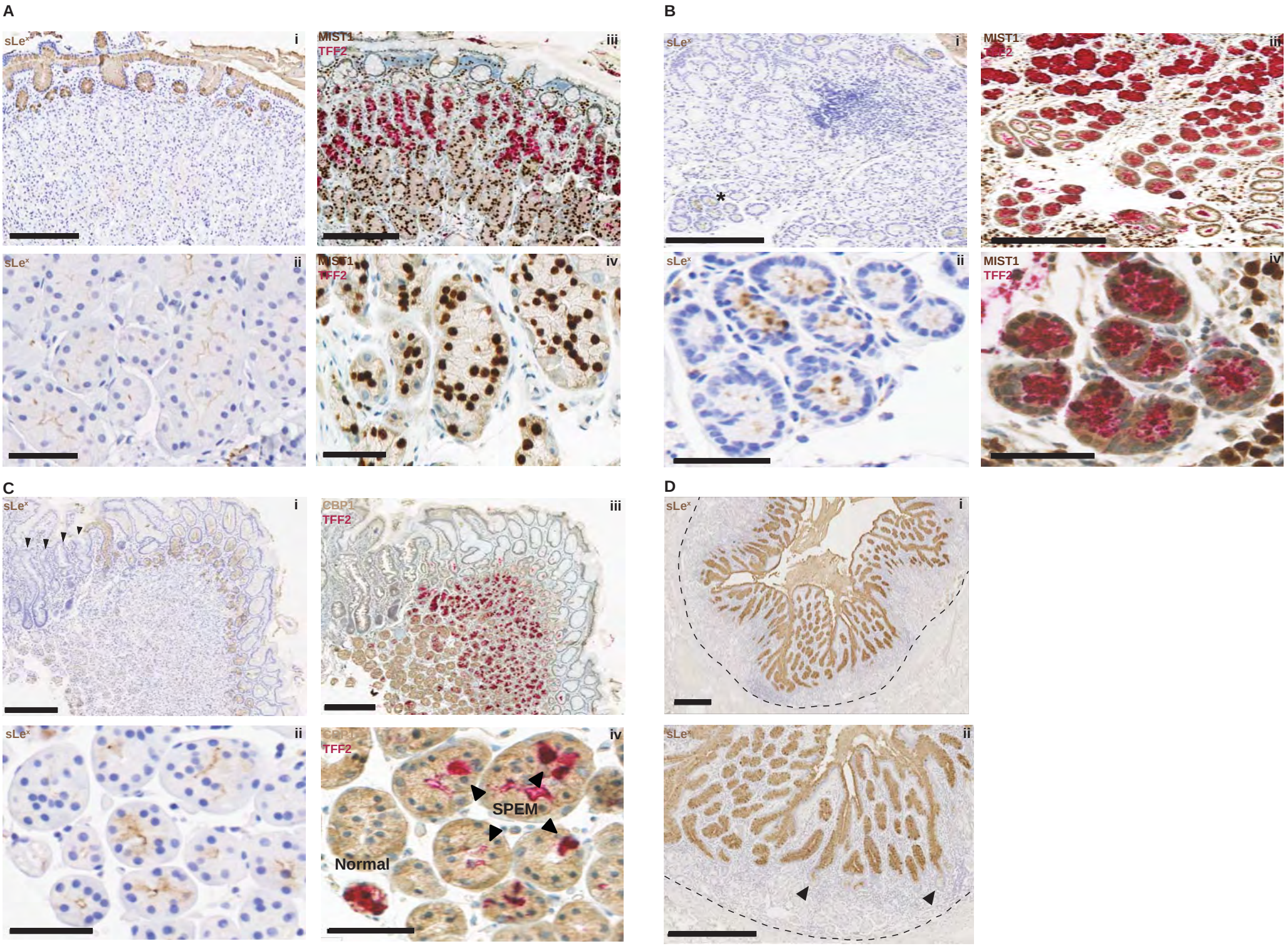
No
(n = 131)

Yes
(n = 7)

Unstained slides or
remaining blocks
available?

No
(n = 2)

Yes
(n = 5)


A
sLe^x
i
MIST1
TFF2
iii
sLe^x
ii
MIST1
TFF2
iv
B
sLe^x
i
*
MIST1
TFF2
iii
sLe^x
ii
MIST1
TFF2
iv
C
sLe^x
i
CBP1
TFF2
iii
sLe^x
ii
CBP1
TFF2
iv
SPEM
Normal
D
sLe^x
i
sLe^x
ii